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The Uptake of Aromatic and Branched Chain Hydrocarbons by Yeast

S. A. Crow*, S. L. Bell and D. G. Ahearn

Laboratory of Microbiology and Biological Chemistry and Department of Biology, Georgia State University, Atlanta, Georgia 30303, USA

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Abstract

Studies of the hydrocarbon utilizing yeasts *Candida maltosa* and *C. lipolytica* have shown that both were capable of reducing recoverable amounts of branched chain and aromatic hydrocarbons in a mixture of naphthalene, tetradecane, hexadecane, pristane (tetra-methylpentadecane). Cells of *C. lipolytica* grown on either glucose or tetradecane were capable of binding (rendering unextractable) nearly 50 % of the hydrocarbon mixture within 3 hours. In contrast cells of *C. maltosa* bound hydrocarbons only after growth on a hydrocarbon medium. Uptake of selected paraffinic hydrocarbon was not altered by the presence of naphthalene. Pristane uptake was concentration dependent for *C. maltosa* but not for *C. lipolytica*. Uptake, transport, and metabolism of hydrocarbons in *C. maltosa* and *C. lipolytica* differ.

Introduction

The ability of yeast to propagate at the expense of single paraffins or simple mixtures of paraffinic hydrocarbons is extensively documented in the literature (Scheda and Bos 1966, Shennan and Levi 1974, Bos 1975). The kinetics of single paraffinic hydrocarbon uptake and assimilation have been studied for a number of the yeast of the genus *Candida* (Munk 1970, Miura *et al.* 1977, Käppeli *et al.* 1978). The processes involved in the transport of alkanes into the cell and their subsequent assimilation are still ill-defined. In general it is agreed that the cell walls of hydrocarbonoclastic yeast possess a physicochemical affinity for hydrocarbons and that they are readily permeable to hydrocarbons. Most studies have dealt with pure alkanes or mixtures of simple alkanes. Hagihara (1977) first reported the utilization of pristane, a branched-chain alkane, as a sole source of carbon and energy by *C. lipolytica*. Crow and Ahearn (in press) have shown that pristane utilization is a common characteristic of isolates of *C. lipolytica*, thus expanding the potential for study of a variety of uptake phenomena. Isolates of both *C. maltosa* and *C. lipolytica* were shown also to be capable of uptake of aromatic and branched-chain hydrocarbons from a broth containing growth supporting n-alkanes. The present study examines the differential uptake of naphthalene and pristane in *C. maltosa* and *C. lipolytica*.

Material and Methods

Cells of *C. lipolytica* (37–1) and *C. maltosa* (R-42) were grown with agitation for 48 hours in yeast nitrogen base broth (YNB) containing 0.1 % glucose. Cells were harvested by centrifugation, washed twice in sterile distilled water and resuspended in water to an optical density of 0.4. This suspension (0.1 ml) was used to inoculate media containing 1.0 % (V/V) of various hydrocarbon mixtures in YNB. The following mixtures were employed: (M1) 0.5 g naphthalene, 0.5 g biphenyl, 10 ml tetradecane, 10 ml hexadecane, and 10 ml pristane; (M2) 1.0 g naphthalene, 10 ml tetradecane, 10 ml hexadecane, and 10 ml of pristane; (M3) 2 without naphthalene; (M4) 1 g naphthalene, 5 ml tetradecane, 5 ml hexadecane, 20 ml pristane. Cultures were incubated on a gyrotary shaker (150 rpm) for 4 days at 22 °C or 12 days at 10 °C. Following incubation, cultures were extracted with 20–50 ml of petroleum ether. A 4 ml aliquot of the petroleum ether extract was removed and placed in a teflon-lined screw cap tube for storage prior to gas-chromatographic analysis. Analyses were performed on a Perkin-Elmer Sigma I Gas Chromatograph

* Person to whom all correspondence should be addressed.

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with FID detectors, fitted with a six-foot stainless steel column packed with 3 % dexsil 300 on chromosorb W. The oven temperature was held at 120 °C for 1 min. The temperature was programmed to 170 °C at 30 °C/min and held for 1 min, and increased to 210 °C at 30 °C/min. Injector temperature was 230 °C, and the detector was held at 250 °C. Flow rate of the carrier gas (nitrogen) was approximately 30 ml/min. All experimental flasks were compared to weathered control flasks; losses of hydrocarbon (accumulation) are reported in μ moles.

The second phase of the study examined the affinity of cells of *C. maltosa* and *C. lipolytica* for individual hydrocarbons and mixtures of hydrocarbons. Cells were grown in Saboraud's dextrose broth for 48 hours and in 0.05 % hexadecane in YNB for 96 hours. The cells were washed three times in sterile distilled water and resuspended to their original volume. Either 0.5 ml of M2 or 0.17 ml of single hydrocarbons were added to 20 ml of the glucose grown cells. This suspension was incubated for 12 hrs at 22 °C with agitation. The hexadecane grown cells were treated similarly but were incubated in the presence of a modified M2 lacking hexadecane at 10 ° and 22 °C for 4 hours. Autoclaved cells from hydrocarbon cultures were added to the hydrocarbon media to evaluate non-specific binding.

Results and Discussion

The utilzation of hydrocarbons by *C. lipolytica* 37–1 and *C. maltosa* R-42 grown in YNB medium with M1 is indicated in Table I. Both yeasts gave good growth and emulsification of the hydrocarbons with preferential utilization of the alkanes. The presence of glucose reduced the utilization of aromatic and branched-chain hydrocarbons by both yeasts and of straight-chain alkanes by *C. maltosa* but not by *C. lipolytica*. Only *C. lipolytica* utilized pristane as a sole source of carbon for growth, but relative uptake of pristane was greatest for *C. maltosa*. However, in the presence of glucose, pristane uptake by *C. maltosa* appeared to be actively depressed, particularly in comparison with the lesser and disproportionate decrease in straight-chain alkane utilization. Moreover, the simultaneous repression of naphthalene and biphenyl utilization by glucose suggested that the yeast transported only readily metabolizable hydrocarbons from a mixture in the presence of glucose.

In YNB medium fortified with M2 both yeasts readily reduced the recoverable amounts of all hydrocarbons at 22 °C (Tab. II). *Candida lipolytica* was more active at 10 °C than *C. maltosa* which was not unexpected due to its psychrotolerant nature and the mesophilic nature of *C. maltosa*. At 10 °C, the accumulation of hydrocarbons by *C. maltosa* was similar to that in the presence of glucose. The readily metabolizable substrates (tetradecane and hexadecane) appear to freely enter the cell to the exclusion of pristane and naphthalene.

The presence of naphthalene did not significantly affect hydrocarbon accumulation by either yeast (Tab. III). In M4 the concentration of pristane, a non-growth supporting compound for *C. maltosa* was twice that used in the other mixtures, and this was reflected by the greater accumulation of pristane by *C. maltosa*. This concentration dependent transport implied that passive transport is functioned in the accumulation of pristane by *C. maltosa*. The mechanism of accumulation in *C. lipolytica* was different since in the presence of higher con-

Tab. I. Utilization of hydrocarbons with growth on MI medium after 10 days at 20 °C

	Hydrocarbons					
Treatment	Naphthalene	Biphenyl	Tetradecane	Hexadecane	Pristane	Total
CONTROL	130[a]	108	1139	1280	973	3630
Candida lipolytica	60[b]	51	1139	1280	583	3113
+ Glucose	42	33	1139	1280	403	3077
Candida maltosa	104	91	1139	1280	840	3454
+ Glucose	13	10	911	1036	155	2125

[a]Micromoles in control
[b]Micromoles utilized

Tab. II. Effect of temperature on hydrocarbon utilization by *Candida lipolytica* and *Candida maltosa*

	Treatment	Hydrocarbons in MI Medium				
		Naphthalene	Pristane	Tetradecane	Hexadecane	Total
Candida maltosa	22 °C	80[a]	361	568	499	1508
	10 °C	7	164	844	604	1619
Candida lipolytica	22 °C	80	397	585	501	1563
	10 °C	134	795	1228	982	3139

[a]Micromoles utilized

centrations of pristane there was no proportionate increase in accumulation of pristane.

The capacity of non-growing yeasts to bind individual hydrocarbons and single components of a hydrocarbon mixture is presented in Table IV. The cell densities for *C. maltosa* were approximately twice those of *C. lipolytica*, yet *C. lipolytica* bound appreciably more hydrocarbons. There was a markedly greater uptake of hexadecane from the water-hydrocarbon mixture than from water-hexadecane system.

The effect of viability and temperature on the binding of hydrocarbons by hydrocarbon grown cells is indicated in Table V. The major difference in binding by equal densities of live and dead cells of *C. maltosa* was the loss of tetradecane binding by dead cells. Live cells held at 10 °C lacked binding capacity for tetradecane and pristane. *C. lipolytica* at a cell density approximately half that of *C. maltosa* bound appreciably more hydrocarbon. Dead cells of *C. lipolytica* and cells at 10 °C bound similar amounts of each hydrocarbon, indicative of non-specific binding. Käppeli (1974) noted increased hydrocarbon binding by hydrocarbon grown cells of *C. tropicalis* due to the presence of increased amounts of lipophilic sites on the walls of hydrocarbon grown cells. He calculated that each cell of an isolate of *C.*

Tab. III. Effect of concentrations of hydrocarbons on hydrocarbon utilization

		Hydrocarbons				
	Treatment	Naphthalene	Pristane	Tetradecane	Hexadecane	Total
Candida maltosa	M2	80[a]	361	568	499	1508
	M3	–	467	627	544	1627
	M4	84	624	304	271	1619
Candida lipolytica	M2	80	397	585	501	1553
	M3	–	436	604	535	1575
	M4	58	407	280	247	992

[a]Micromoles utilized; –, component not present

Tab. IV. Uptake of hydrocarbons (μM) by glucose grown cells of *Candida maltosa* and *Candida lipolytica* after 12 hours

		Hydrocarbons			
	Treatment	Naphthalene	Pristane	Tetradecane	Hexadecane
Candida maltosa	Control	130[a]	485[a]	640[a]	564[a]
	M2	65[b]	111	57	22
	Pristane	–	34	–	–
	Tetradecane	–	–	121	–
	Hexadecane	–	–	–	6
Candida lipolytica	M2	117	398	492	423
	Pristane	–	320	–	–
	Tetradecane	–	–	510	–
	Hexadecane	–	–	–	186

[a]Micromoles recovered in control systems
[b]Micromoles bound in experimental systems; –, component not present

Tab. V. Uptake of hydrocarbons (μM) by hydrocarbon grown cells of *Candida maltosa* and *Candida lipolytica* after 4 hours

		Hydrocarbons		
	Treatment	Naphthalene (7.95)[a]	Pristane (32.5)	Tetradecane (42.8)
Candida maltosa	25°C	1.59[b]	3.57	7.04
	10 °C	1.27	0.74	1.07
	Autoclaved	2.06	3.57	1.57
Candida lipolytica	25 °C	3.29	16.52	21.82
	10 °C	3.34	9.97	13.16
	Autoclaved	3.43	9.11	12.20

[a]Micromoles recovered from control
[b]Micromoles bound in experimental systems

tropicalis bound a layer of hydrocarbon 0.08 μm in thickness. Our calculations indicate that hexadecane-grown cells suspended in hydrocarbons other than hexadecane under non-growth conditions become coated by a layer approximately 0.02 μm in thickness. The different growth rates for *C. maltosa* and *C. lipolytica* grown on hydrocarbons and their different patterns of hydrocarbon uptake suggest that systems additional to non-specific binding may be operating in these yeasts. Further studies are needed to determine the kinetics of uptake and accumulation of aromatic hydrocarbons from complex mixture of hydrocarbons by yeasts.

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